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TITLE: Breast Cancer Prevention by Inducing Apoptosis in DCIS Using Breast Ductal Lavage

PRINCIPAL INVESTIGATOR: Patrick P. Koty, Ph.D.

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, PA 15260

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6. AUTHOR(S)

Patrick P. Koty, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Pittsburgh
Pittsburgh, PA 15260

E-Mail: pkoty@wfubmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. ABSTRACT (Maximum 200 Words)

Although great strides have been made in breast cancer screening and treatment, it remains the second highest cause of cancer-related deaths for women in the United States. Current prevention focuses on oral administration of tamoxifen which decreases breast cancer incidence but increases the risk for secondary uterine cancer. In addition, tamoxifen may not be effective in preventing those lesions that are estrogen receptor (ER) negative based on its primary function of suppressing cell proliferation by blocking the estrogen receptor. We hypothesize that programmed cell death is dysregulated in premalignant and malignant breast cells which permits both ER-positive and ER-negative cells to avoid cell death. We intend to investigate whether treating premalignant breast cells with a molecular genetic-based agent may be effective alone or in concert with tamoxifen treatment to induce cell death in both ER-positive and ER-negative cells. Ultimately, we envision delivering genetic-based preventive agents and/or tamoxifen directly to the breast ductal lobe of these high risk individuals thus eliminating any potential for tamoxifen-induced uterine cancers.

14. SUBJECT TERMS
Chemoprevention, apoptosis, breast ductal lavage, ductal carcinoma in situ., bcl-2 gene family, gene therapy, transfection

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Table of Contents

TITLE:

Breast Cancer Prevention by Inducing Apoptosis in DCIS Using Breast Ductal Lavage

INTRODUCTION:

Although great strides have been made in breast cancer screening and treatment, it remains the second highest cause of cancer-related deaths for women in the United States. Current prevention has focused on oral administration of tamoxifen which appears to decrease breast cancer incidence but increases the risk for secondary uterine cancer. In addition, tamoxifen may not be effective in preventing those lesions that are estrogen receptor (ER) negative based on its primary function of suppressing cell proliferation by blocking the estrogen receptor. We hypothesize that programmed cell death (PCD) is dysregulated in premalignant and malignant breast cells which permits both ER-positive and ER-negative cells to avoid cell death. We intend to investigate whether treating premalignant breast cells with a molecular genetic-based agent (antisense bcl-2/bcl-xL oligonucleotide) may be effective alone or in concert with tamoxifen treatment to induce cell death in both ER-positive and ER-negative cells. Ultimately, we envision using the newly developed technique of breast ductal lavage to not only screen women for increased risk in developing breast cancer, which is currently being performed, but to also use this technique to delivery genetic-based preventive agents and/or tamoxifen directly to the breast ductal lobe of these high risk individuals thus eliminating any potential for tamoxifen-induced uterine cancers.

BODY:

The following is the approved Statement of Work for the project entitled *"Breast Cancer Prevention by Inducing Apoptosis in DCIS Using Breast Ductal Lavage."* When the Award was granted, the terms indicated that no patient samples could be obtained until the DoD's Human Subject Protection Office approved this project for the Use of Human Anatomical Substances. This still has not occurred, even though, this project received IRB approval on October 14, 2002 from the institution (Magee Womens Hospital, Pittsburgh PA) for which the samples were to be obtained. The documentation requested by the Human Subject Protection Office was sent to Ms. Catherine Smith on October 21, 2002 then transferred to Ms. Robin Dillner on November 5, 2002 but no decision on approval status was ever given.

On April 1, 2003 I moved my research laboratory from the University of Pittsburgh, Pittsburgh PA to Wake Forest University School of Medicine, Winston-Salem NC. Since being here, I received IRB approval (October 9, 2003) from Magee Womens Hospital, Pittsburgh PA to send any future patient samples to my research laboratory at Wake Forest University School of Medicine. In addition, I have received IRB approval (April 1, 2004) to not only accept samples from Magee Womens Hospital but to obtain additional ductal lavage samples from Wake Forest University School of Medicine. On May 18, 2004 I submitted all the requested documents to Ms. Patricia Dubill from the DoD's Human Subject Protection Office. I received a response from Ms. Dubill on July 15, 2004 regarding this submission indicating a need for further clarification and modification. I am currently working on these issues and will resubmit shortly. Therefore, without DoD's Human Subject Protection Office's approval for this project

for the Use of Human Anatomical Substances I can not perform any research until I receive such approval as indicated on the terms of the subject award.

In addition, Ms. Barbara McMullen from the University of Pittsburgh notified Wake Forest University School of Medicine on August 26, 2004 that they have not released this grant funding in contradiction to an email I received indicating the funds were released for this project on August 13, 2003. Ms. McMullen is currently awaiting procedural instructions from Ms. Brenda Merson (Contract Specialist – Grants Transfer Section – USAMRAA) to release the funds.

Given these delays, I have recently embarked on initiating this project using very limited internal funding. Beginning August 10, 2004, we have been able to obtain inform consent from four patients recruited through the Wake Forest School of Medicine. Breast ductal lavage samples were obtained from three of the four patients.. We are currently evaluating these ductal lavage samples using protocols designed to accomplish *Task 3* in the following "Statement of Work". In addition, after negotiating a consultant contract, Dr. Jean Latimer should be supplying the five DCIS primary explant cultures and normal mammary tissue controls that were originally proposed to be used to accomplish *Task 1* and *Task 2* in the following "Statement of Work".

After receiving the DoD's Human Subject Protection Office's approval for this project for the Use of Human Anatomical Substances and transferring the funds for this project to Wake Forest University School of Medicine, I will request a no-cost extension to complete the project in an appropriate time frame.

Statement of Work

Task 1. Determine expression pattern of the PCD regulatory genes bcl-2, bax, and bcl-xL in primary DCIS cultures (Months 1-6):

a. Protein analysis of Bcl-2, Bax, and Bcl-xL using Western blotting and immunofluorescent staining.

Task 2. Determine whether down-regulation by genetic manipulation of the anti-apoptotic genes bcl-2 and/or bcl-xL alone or in conjunction with physiological preventive doses of tamoxifen has the highest induction of PCD in primary DCIS cell cultures (Months 6-18):

a. Treatment with antisense and control oligonucleotides and/or tamoxifen.
b. Protein analysis of Bcl-2, Bax, and Bcl-xL using Western blotting and immunofluorescent staining.
c. Quantify mRNA for bcl-2 or bcl-xL using a PCR-based assay.
d. Determine effect of treatment on programmed cell death markers using assays for DNA fragmentation and caspase activation.

Task 3. Determine expression pattern of the PCD regulatory genes bcl-2, bax, and bcl-xL in cells obtained by breast ductal lavage (Months 18-24):

a. Protein analysis of Bcl-2, Bax, and Bcl-xL using immunofluorescent staining.

Task 4. Determine whether down-regulation by genetic manipulation of the anti-apoptotic genes bcl-2 and/or bcl-xL alone or in conjunction with physiological

preventive doses of tamoxifen has the highest induction of PCD in cells from breast ductal lavages (Months 24-36).

a. Treatment with antisense and control oligonucleotides and/or tamoxifen.
b. Protein analysis of Bcl-2, Bax, and Bcl-xL using Western blotting and immunofluorescent staining.

c. Quantify mRNA for bcl-2 or bcl-xL using a PCR-based assay.
d. Determine effect of treatment on programmed cell death markers using assays for DNA fragmentation and caspase activation.

KEY RESEARCH ACCOMPLISHMENTS: N/A

REPORTABLE OUTCOMES: N/A

CONCLUSIONS: N/A

REFERENCES: N/A

APPENDICES: N/A